Chemical Constituents from *Myrsine africana* L.

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Three new compounds, myrsinoside A (=2,4-dihydroxy-6-methylphenyl β-D-(6′-galloyl)glucopyranoside; **1**), myrsinoside B (2,4-dihydroxy-6-methylphenyl β-D-glucopyranoside; **2**), and (3β,16α,20α)-3,16,28-trihydroxyolean-12-en-29-oic acid 3-{*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranoside} (**3**), along with four known compounds, were isolated from the stems of *Myrsine africana* L. The structures of these new compounds were elucidated on the basis of spectroscopic analysis, including 1D- and 2D-NMR and ESI-MS techniques, and chemical methods.

Introduction. – *Myrsine africana* L. is a plant of the Myrsinaceae distributed widely from the Himalayas, China, and the Azores to eastern and southern Africa [1]. It has been used as a traditional Chinese medicine for the treatment of diarrhea, rheumatism, toothache, and pulmonary tuberculosis and for relieving hemorrhage [2]. Previous studies on this plant revealed the presences of flavonoids [3][4][5], benzoquinones [6][7], and triterpenoids [8]. Our phytochemical investigation of the 95% EtOH extract of stems of *M. africana* L. resulted in the isolation of seven compounds, including three new ones. In this article, we report the isolation and structural elucidation of the new compounds.

Results and Discussion. – The dried and powdered stems of *M. africana* L. were extracted with 95% EtOH. After evaporation, the extract was suspended in H_2O and partitioned successively with petroleum ether, $CHCl_3$, AcOEt, and BuOH. After purification by repeated chromatographic procedures, the AcOEt-soluble fraction afforded myrsinosides A[1]) (**1**) and B (**2**), together with gallic acid (=3,4,5-trihydroxybenzoic acid) [9], and the BuOH-soluble fraction gave a new triterpene glycoside **3** (*Fig.*), along with three known lignan glycosides isolariciresinol 9′-β-D-xylopyranoside [10], isolariciresinol 9′-β-D-glucopyranoside [11], and lyoniresinol 9′-β-D-glucopyranoside [12] (isolariciresinol = (1*S*,2*R*,3*R*)-1,2,3,4-tetrahydro-7-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-6-methoxynaphthalene-2,3-dimethanol; lyoniresinol = (1*S*,2*R*,3*R*)-1,2,3,4-tetrahydro-7-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-6,8-dimethoxynaphthalene-2,3-dimethanol).

Myrsinoside A (**1**) was obtained as a white amorphous powder. Its molecular formula $C_{20}H_{22}O_{12}$ was deduced from the HR-ESI-MS (*m*/*z* 477.0997 ($[M+Na]^+$)). Acid hydrolysis of **1** gave D-glucose as sugar moiety. The IR bands at 1697, 3376, 1612,

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

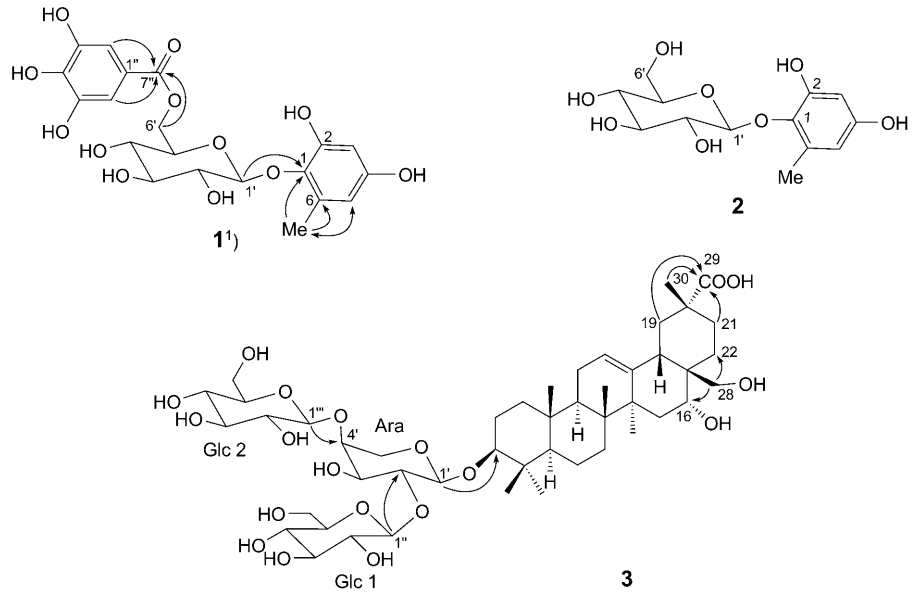

Figure. *The structures of* **1**–**3** *and selective HMBCs* (H → C) *of* **1** *and* **3**

and 1450 cm^{-1} suggested the presence of C=O, OH, and aromatic groups in the molecule of **1**. The analysis of ^{1}H- and ^{13}C-NMR and HMBC spectra (*Table 1* and *Fig.*) allowed us to elucidate the structure of **1** to be 2,4-dihydroxy-6-methylphenyl 6-*O*-galloyl-*β*-D-glucopyranoside.

The ^{1}H- and ^{13}C-NMR spectra of **1** showed signals for one galloyl (δ(H) 7.08 (*s*, 2 H); δ(C) 168.8, 147.0 (2 C), 140.4, 121.8, and 110.7 (2 C)), one trioxy- and methyl-substituted aromatic ring (δ(H) 6.14 and 6.04 (2*d*, each $J = 2.4$ Hz, each 1 H) and 2.17 (*s*, 3 H); δ(C) 156.1, 151.8, 139.3, 134.7, 109.5, 102.5, and 17.7 (*q*)), and one *β*-glucopyranosyl residue (δ(H) 4.46 (*d*, $J = 7.6$ Hz, 1 H); δ(C) 108.5, 78.3, 76.5, 76.0, 72.0, and 65.1). The substitutents at the aromatic ring were assigned to be 1,2,4-trioxy and 6-methyl based on the HMBC (*Fig.*) between Me–C(6) and C(1), C(5), and C(6). Furthermore, the connectivities of C(1)–O–C(1′) and C(6′)–O–*C*(7″) were established by the HMBC (*Fig.*) cross-peaks H–C(1′)/C(1) and CH_2(6′)/C(7″).

Myrsinoside B (**2**), a white amorphous powder, had the molecular formula $C_{13}H_{18}O_8$ as determined by the HR-ESI-MS (*m*/*z* 325.0882 ($[M + Na]^+$)). The acid hydrolysis of **2** afforded D-glucose. Its ^{1}H- and ^{13}C-NMR spectra were almost superposable with those of **1**, except for the absence of the galloyl group and an upfield shift of the C(6′) signal (from δ(C) 65.1 of **1** to 62.8 of **2**), which indicated the structure of **2** to be 6′-*O*-degalloylmyrsinoside A, *i.e.*, 2,4-dihydroxy-6-methylphenyl *β*-D-glucopyranoside.

Compound **3**, a white amorphous powder, was assigned the molecular formula $C_{47}H_{76}O_{19}$ based on the quasimolecular-ion peak at *m*/*z* 967.4841 ($[M + Na]^+$, $C_{47}H_{76}O_{19}Na^+$) in the HR-ESI-MS. The other main ESI-MS fragments at *m*/*z* 783 ($[M + H - 162]^+$), 621 ($[M + H - 162 - 162]^+$), and 489 ($[M + H - 162 - 162 - 132]^+$) suggested the existence of two hexoses and one pentose unit in the molecule of **3**, which

Table 1. *^{1}H- and ^{13}C-NMR Data* (CD_3OD; 400 and 100 MHz, resp.) *of* **1**[1]) *and* **2**. δ in ppm, J in Hz.

	δ(H)		δ(C)	
	1	**2**	**1**	**2**
C(1)			139.3	139.2
C(2)			151.8	151.9
H–C(3) or C(3)	6.14 (*d*, *J* = 2.4)	6.15 (*d*, *J* = 2.7)	102.5	102.6
C(4)			156.1	156.0
H–C(5) or C(5)	6.04 (*d*, *J* = 2.4)	6.09 (*d*, *J* = 2.4)	109.5	109.5
C(6)			134.7	134.5
Me–C(6)	2.17 (*s*)	2.21 (*s*)	17.7	17.8
Glc:				
H–C(1′) or C(1′)	4.46 (*d*, *J* = 7.6)	4.45 (*d*, *J* = 7.6)	108.5	108.4
H–C(2′) or C(2′)	3.46 – 3.52 (*m*)	3.49 – 3.56 (*m*)	76.0	75.9
H–C(3′) or C(3′)	3.42 – 3.48 (*m*)	3.44 – 3.50 (*m*)	78.3	78.5
H–C(4′) or C(4′)	3.45 – 3.49 (*m*)	3.47 – 3.55 (*m*)	72.0	71.5
H–C(5′) or C(5′)	3.50 – 3.57 (*m*)	3.24 – 3.28 (*m*)	76.5	78.8
CH_2(6′) or C(6′)	4.55 (*dd*, *J* = 11.7, 2.1), 4.44 (*dd*, *J* = 11.7, 6.0)	3.86 (*dd*, *J* = 12.0, 2.4), 3.75 (*dd*, *J* = 12.0, 5.7)	65.1	62.8
Galloyl:				
C(1″)			121.8	
H–C(2″) or C(2″)	7.08 (*s*)		110.7	
C(3″)			147.0	
C(4″)			140.4	
C(5″)			147.0	
H–C(6″) or C(6″)	7.08 (*s*)		110.7	
C(7″)			168.8	

were established to be D-glucose and L-arabinose by acidic hydrolysis experiments. The detailed analysis of the 1D- and 2D-NMR data, as well as comparison of the NMR data of **3** with those of saponins of the oleanolic acid type such as ardisicrenoside G[2]) [13], triptocallic acid D[2]) [14], and davuricoside H[2]) [15], elucidated the structure of **3** to be (3β,16α,20α)-3,16,28-trihydroxyolean-12-en-29-oic acid 3-{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 4)]-α-L-arabinopyranoside}.

The ^{1}H-NMR spectrum of **3** displayed signals for three anomeric H-atoms (δ(H) 4.63 (*d*, *J* = 7.8 Hz), 4.51 (*d*, *J* = 5.6 Hz), and 4.48 (*d*, *J* = 7.8 Hz)), in agreement with the configurations of α-arabinopyranosyl and β-glucopyranosyl units. The other ^{1}H-NMR signals for the aglycone were attributed to six Me groups (δ(H) 1.40, 1.22, 1.06, 0.98, 0.95, and 0.86), one CH_2OH group (δ(H) 3.25 and 3.10 (2*d*, each *J* = 7.2 Hz, each 1 H)), one olefinic H-atom (δ(H) 5.31 (br. *s*)), and two oxygenated CH groups (δ(H) 3.12 – 3.17 and 3.85 – 3.89 (2*m*)). The ^{13}C-NMR spectrum (*Table 2*) exhibited 30 C-signals for the aglycone, which were resolved into 6 Me, 10 CH_2, 6 CH, 6 C_q, and 2 C (sp^2). The above evidences suggested that **3** was a trioxylated oleanolic acid type triterpenoid saponin [13][14][15]. The COOH group was assigned to C(29) by comparison of the ^{13}C-NMR data of the Me groups of **3** with those of ardisicrenoside G[2]) [13],

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[2]) Ardisicrenoside G = (3β,16α,20β)-3-{{*O*-6-deoxy-α-L-mannopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranosyl}oxy}-16,28-dihydroxyolean-12-en-29-oic acid; triptocallic acid D = (3α,20α,22α)-3,22-dihydroxyolean-12-en-29-oic acid; davuricoside H = (3β,16α,20β)-3-{{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 4)]-α-L-arabinopyranosyl}oxy}-16,28-dihydroxyolean-12-en-29-oic acid.

triptocallic acid D[2]) [14], and davuricoside H[2]) [15], which was further confirmed by the ROESY correlation between Me–C(20) and H–C(18). The three oxy groups of **3** were positioned at C(3)(β), C(16)(α), and C(28), according to the ^{1}H-NMR coupling constants and comparison of the ^{13}C-NMR data with those of triptocallic acid D and davuricoside H [15].

Table 2. *^{13}C-NMR Data* (CD_3OD; 100 MHz) *of* **3**. δ in ppm.

	δ(C)		δ(C)		δ(C)		δ(C)
C(1)	40.5	C(14)	41.8	C(27)	28.1	C(3″)	78.4
C(2)	27.5	C(15)	35.4	C(28)	71.0	C(4″)	72.0
C(3)	91.8	C(16)	73.8	C(29)	183.9	C(5″)	78.5
C(4)	40.9	C(17)	41.5	C(30)	21.4	C(6″)	63.2
C(5)	57.5	C(18)	42.7	Ara:		Glc 2:	
C(6)	19.8	C(19)	43.3	C(1′)	105.6	C(1‴)	106.1
C(7)	34.5	C(20)	43.1	C(2′)	79.2	C(2‴)	75.9
C(8)	43.8	C(21)	32.5	C(3′)	75.1	C(3‴)	78.6
C(9)	48.6	C(22)	29.3	C(4′)	78.8	C(4‴)	72.4
C(10)	38.3	C(23)	29.1	C(5′)	65.0	C(5‴)	78.5
C(11)	25.1	C(24)	17.4	Glc 1:		C(6‴)	63.6
C(12)	124.6	C(25)	16.7	C(1″)	105.0		
C(13)	145.1	C(26)	18.1	C(2″)	76.3		

The glycone unit was assigned to β-D-Glc-(1→2)-[β-D-Glc-(1→4)]-α-L-Ara on the basis of the similarity of the NMR data with those of davuricoside H [15] and the HMBC cross-peaks H–C(1′)/C(3), H–C(1″)/C(2′), and H–C(1‴)/C(4′) (*Fig.*).

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 or 400 mesh; *Qingdao Haiyang, Co.*, P. R. China), *ODS-A* gel (*Greenherbs Science and Technology Development Co., Ltd.*, Beijing, P. R. China), *D-1400* macroporous resin (*Yangzhou Pharmaceutical Factory*, P. R. China), and *Sephadex LH-20* (*Pharmacia Biotech AB*, Uppsala, Sweden). GC: *Perkin-Elmer Sigma-115* gas chromatograph. Optical rotation: *Perkin-Elmer 341* polarimeter. IR Spectra: *Nicolet Magna-750-FTIR* spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker DRX-400* instrument; at 400 (^{1}H) or 100 MHz (^{13}C); in CD_3OD; δ in ppm rel. to Me_4Si, *J* in Hz. ESI-MS and HR-ESI-MS: *LCQ Deca* and *Q-Tof Ultima* mass spectrometers, resp.; in *m*/*z*.

Plant Material. The stems of *M. africana* L. were collected in March 2005 from Dali of Yunnan Province, P. R. China, and was authenticated by Dr. *J. Huang* of our institute. A voucher specimen (No. 20050308) was deposited with the Herbarium of our institute.

Extraction and Isolation. The dried and powdered stems of *M. africana* L. (6 kg) were extracted with 95% EtOH (3 × 50 l) by maceration for 48 h. The solvent was evaporated, and the residue (650 g) was suspended in H_2O and then partitioned successively with petroleum ether, $CHCl_3$, AcOEt, and BuOH. The AcOEt-soluble part was subjected to CC (SiO_2 (2 kg), $CHCl_3$/MeOH of increasing polarity): *Fractions 1–7. Fr. 4* afforded gallic acid (786 mg) after purification by two CCs (1. *Sephadex LH-20*, MeOH; 2. *ODS*, MeOH/H_2O 20:80). *Fr. 5* yielded **2** (315 mg) after three CCs (1. SiO_2, $CHCl_3$/MeOH 8:1; 2. *ODS-A* gel, MeOH/H_2O 20:80; 3. *Sephadex LH-20*, MeOH). Compound **1** (30 mg) was obtained from *Fr. 6* after purification by three CCs (1. SiO_2, $CHCl_3$/MeOH 8:1; 2. *ODS-A*, MeOH/H_2O 15:85; 3. *Sephadex LH-20*, MeOH/H_2O 70:30). The BuOH-soluble part (155 g) was subjected to CC (macroporous resin (i.d. 10 × 80 cm), EtOH/H_2O 0:100, 10:90, 30:70, 50:50, 70:30, and 95:5): *Frs. A–F. Fr. C* (with 30% EtOH; 32.5 g) was separated by CC (SiO_2, $CHCl_3$/MeOH 20:1 → 0:1): *Frs. C1–C8*. *Fr. C2* afforded isolariciresinol 9′-β-D-xylopyranoside (12 mg), isolariciresinol 9′-β-D-glucopyranoside (55 mg),

and lyoniresinol 9′-β-D-glucopyranoside (80 mg) after CCs (SiO_2, $CHCl_3$/MeOH 10:1); *Sephadex LH-20*, MeOH/H_2O 80:20; *ODS*, MeOH/H_2O 15:85 for isolariciresinol 9′-β-D-glucopyranoside, 25:75 for lyoniresinol 9′-β-D-glucopyranoside, 35:65 for isolariciresinol 9′-β-D-xylopyranoside). *Fr. C5* furnished **3** (12 mg) by three CCs (1. SiO_2, $CHCl_3$/MeOH/H_2O 5:2:0.05; 2. *Sephadex*, MeOH/H_2O 80:20; 3. *ODS*, MeOH/H_2O 20:80).

Myrsinoside A (=*2,4-Dihydroxy-6-methylphenyl β-D-Glucopyranoside 6-(3,4,5-Trihydroxybenzoate)*; **1**): White amorphous powder. $[\alpha]_D^{22} = -54.2$ ($c = 0.345$, MeOH). IR: 3376, 2931, 1697, 1612, 1450. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS (pos.; neg.): 477 ($[M+Na]^+$); 453 ($[M-H]^-$), 907 ($[2M-H]^-$). HR-ESI-MS: 477.0997 ($[M+Na]^+$, $C_{20}H_{22}NaO_{12}^+$; calc. 477.1009).

Myrsinoside B (=*2,4-Dihydroxy-6-methylphenyl β-D-Glucopyranoside*; **2**): White amorphous powder. $[\alpha]_D^{22} = -15.1$ ($c = 0.345$, MeOH). IR: 3386, 2923, 1606, 1498. ^{1}H- and ^{13}C- NMR: *Table 1*. ESI-MS (pos.; neg.): 325 ($[M+Na]^+$); 301 ($[M-H]^-$), 139 ($[M-H-162]^-$). HR-ESI-MS: 325.0882 ($[M+Na]^+$, $C_{13}H_{18}NaO_8^+$; calc. 325.0899).

(3β,16α,20α)-3,16,28-Trihydroxyolean-12-en-29-oic Acid 3-{O-β-D-Glucopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranoside} (=*(3β,16α,20α)-3-{{O-β-D-Glucopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl}oxy}-16,28-dihydroxyolean-12-en-29-oic Acid*; **3**): White amorphous powder. $[\alpha]_D^{22} = -5.8$ ($c = 0.325$, MeOH). IR: 3415, 2925, 1700, 1639, 1465. ^{1}H-NMR: 5.31 (br. *s*, H–C(12)); 4.63 (*d*, *J* = 7.6, H–C(1″)); 4.51 (*d*, *J* = 5.6, H–C(1′)); 4.48 (*d*, *J* = 7.6, H–C(1‴)); 4.15 (*dd*, *J* = 12.0, 4.0, H–C(5′)); 3.99–3.94 (*m*, H–C(4′)); 3.95–3.92 (*m*, H–C(3′)); 3.94–3.88 (*m*, H–C(2′)); 3.89–3.85 (*m*, H–C(16)); 3.88–3.80 (*m*, H–C(6″), H–C(6‴)); 3.70–3.57 (*m*, H–C(6″), H–C(6‴)); 3.59–3.51 (*m*, H–C(5′)); 3.39–3.32 (*m*, H–C(5″)); 3.31–3.23 (*m*, H–C(3‴), H–C(5‴), H–C(3″)); 3.29–3.25 (*m*, H–C(4″), H–C(4‴)); 3.26–3.20 (*m*, H–C(2″), H–C(2‴)); 3.25 (*d*, *J* = 7.2, H–C(28)); 3.17–3.12 (*m*, H–C(3)); 3.10 (*d*, *J* = 7.2, H–C(28)); 2.68 (*t*, *J* = 13.2, H–C(19)); 2.36 (*m*, H–C(21)); 2.10 (*dd*, *J* = 14.0, 3.6, H–C(18)); 1.95–1.87 (*m*, H–C(15)); 1.94–1.86 (*m*, H–C(11)); 1.87–1.79 (*m*, H–C(2)); 1.85–1.76 (*m*, H–C(22)); 1.80–1.72 (*m*, H–C(2)); 1.68–1.61 (*m*, H–C(22), H–C(9), H–C(1)); 1.61–1.53 (*m*, H–C(6)); 1.61–1.57 (*m*, H–C(7)); 1.58–1.55 (*m*, H–C(11)); 1.46–1.39 (*m*, H–C(21)); 1.44–1.40 (*m*, H–C(6)); 1.42–1.36 (*m*, H–C(7)); 1.40 (*s*, Me(27)); 1.38–1.29 (*m*, H–C(15)); 1.36–1.28 (*m*, H–C(19)); 1.22 (*s*, Me(30)); 1.06 (*s*, Me(23)); 1.04–0.97 (*m*, H–C(1)); 0.98 (*s*, Me(25)); 0.95 (*s*, Me(26)); 0.86 (*s*, Me(24)); 0.81 (*dd*, *J* = 8.2, 1.6, H–C(5)). ^{13}C-NMR: *Table 2*.

Acid Hydrolysis of **1**–**3**. Acid hydrolysis of **1**–**3** and sugar identification were conducted according to our standard procedure [16]. In brief, each glycoside (*ca.* 2.0 mg) in 2N HCl/dioxane 1:1 (2 ml) was refluxed for 2 h. On cooling, the mixture was neutralized with $NaHCO_3$. After extraction with AcOEt, the aq. layer was concentrated by blowing with N_2. The residue was purified by CC (*Sephadex LH-20*, MeOH/H_2O 1:1) to give the sugar mixture. The purified sugar and standard D-glucose and L-arabinose (*Sigma*, USA) were treated with L-cysteine methyl ester hydrochloride (2 mg) in pyridine (1 ml) at 60° for 1 h. Then, the soln. was treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (0.02 ml) at 60° for 1 h. The supernatant was applied to GLC analysis (*Supelco*; 230°, N_2). D-Glucose (t_R 24.0 min) was detected from **1**–**3**, and L-arabinose (t_R 12.1 min) from **3** by comparing their retention times with the authentic samples.

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